

**Figure 1**

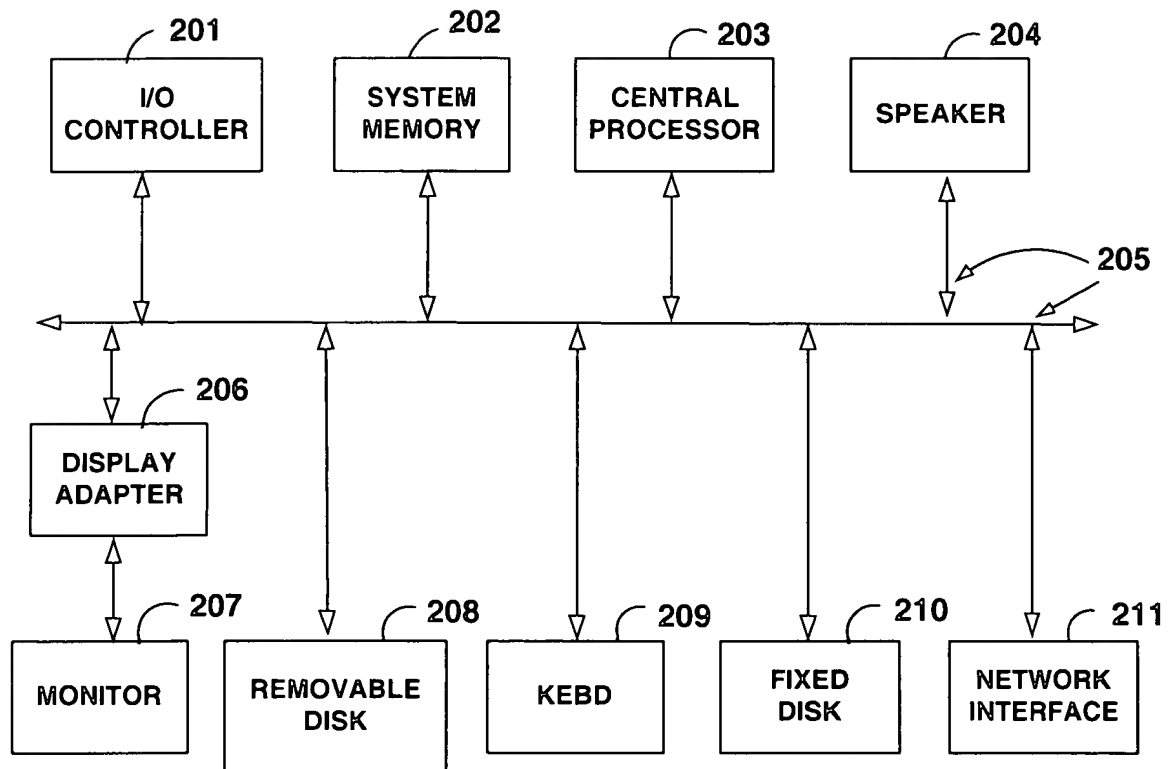
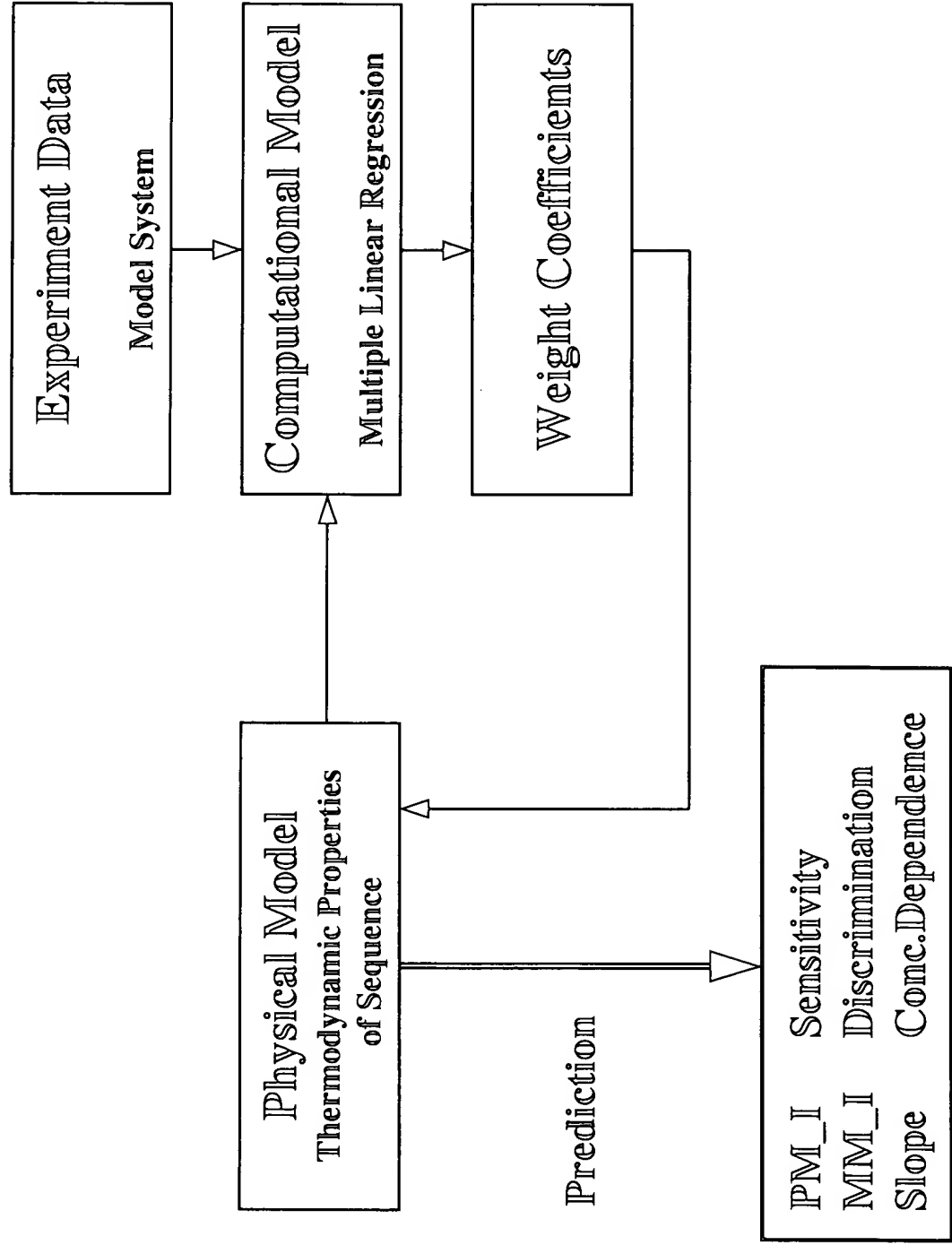


Figure 2

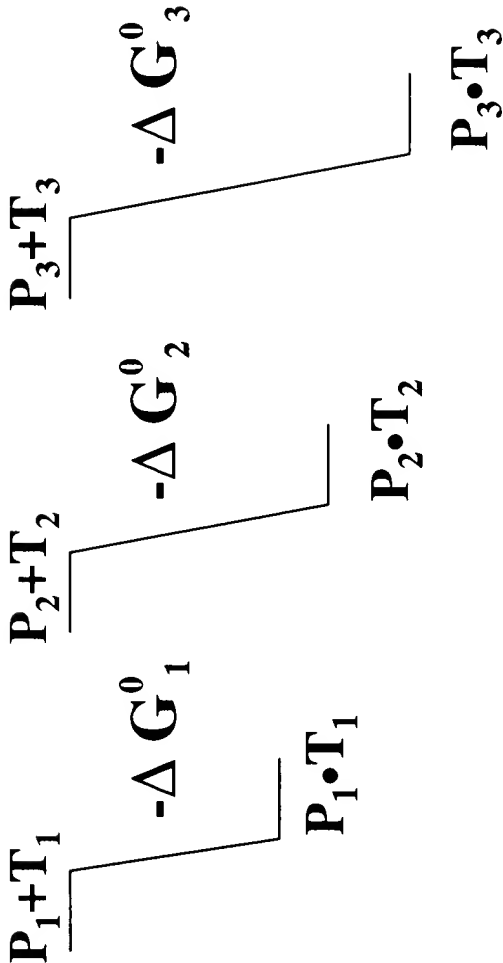
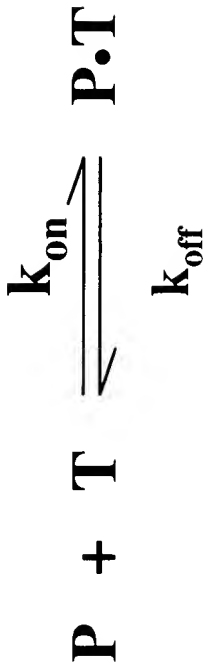
001221" 59654260  
Predicting Probe Quality

Figure 3



# Basic Physical Model

Figure 4



|   | 1 | 2 | 3 | 4 | 5 | 6 | 7 | 8 | 9 | 10 | 11 | 12 | 13 | 14 | 15 | 16 | 17 | 18 | 19 | 20 | 21 | 22 | 23 | 24 | 25 | 26 | 27 | 28 | 29 | 30 | 31 | 32 | 33 | 34 | 35 | 36 | 37 | 38 | 39 | 40 | 41 | 42 | 43 | 44 | 45 | 46 | 47 | 48 | 49 | 50 | 51 | 52 | 53 | 54 | 55 | 56 | 57 | 58 | 59 | 60 | 61 | 62 | 63 | 64 | 65 | 66 | 67 | 68 | 69 | 70 | 71 | 72 | 73 | 74 | 75 | 76 | 77 | 78 | 79 | 80 | 81 | 82 | 83 | 84 | 85 | 86 | 87 | 88 | 89 | 90 | 91 | 92 | 93 | 94 | 95 | 96 | 97 | 98 | 99 | 100 |
|---|---|---|---|---|---|---|---|---|---|----|----|----|----|----|----|----|----|----|----|----|----|----|----|----|----|----|----|----|----|----|----|----|----|----|----|----|----|----|----|----|----|----|----|----|----|----|----|----|----|----|----|----|----|----|----|----|----|----|----|----|----|----|----|----|----|----|----|----|----|----|----|----|----|----|----|----|----|----|----|----|----|----|----|----|----|----|----|----|----|----|----|----|----|----|----|----|----|----|----|-----|
| 1 | 1 | 2 | 3 | 4 | 5 | 6 | 7 | 8 | 9 | 10 | 11 | 12 | 13 | 14 | 15 | 16 | 17 | 18 | 19 | 20 | 21 | 22 | 23 | 24 | 25 | 26 | 27 | 28 | 29 | 30 | 31 | 32 | 33 | 34 | 35 | 36 | 37 | 38 | 39 | 40 | 41 | 42 | 43 | 44 | 45 | 46 | 47 | 48 | 49 | 50 | 51 | 52 | 53 | 54 | 55 | 56 | 57 | 58 | 59 | 60 | 61 | 62 | 63 | 64 | 65 | 66 | 67 | 68 | 69 | 70 | 71 | 72 | 73 | 74 | 75 | 76 | 77 | 78 | 79 | 80 | 81 | 82 | 83 | 84 | 85 | 86 | 87 | 88 | 89 | 90 | 91 | 92 | 93 | 94 | 95 | 96 | 97 | 98 | 99 | 100 |

## Figure 5

## Example : GTCA

\*Using A as ref. 3 base/position

| <u>i</u>                        | <u>Position</u> | <u>Base</u> | <u>S<sub>i</sub></u> |
|---------------------------------|-----------------|-------------|----------------------|
| 1                               | 1               | C           | 0                    |
| 2                               | 1               | G           | 1                    |
| 3                               | 1               | T           | 0                    |
| (1 <sup>st</sup> position is G) |                 |             |                      |
| 4                               | 2               | C           | 0                    |
| 5                               | 2               | G           | 0                    |
| 6                               | 2               | T           | 1                    |
| (2 <sup>nd</sup> position is T) |                 |             |                      |
| 7                               | 3               | C           | 1                    |
| 8                               | 3               | G           | 0                    |
| 9                               | 3               | T           | 0                    |
| (3 <sup>rd</sup> position is C) |                 |             |                      |
| 10                              | 4               | C           | 0                    |
| 11                              | 4               | G           | 0                    |
| 12                              | 4               | T           | 0                    |
| (4 <sup>th</sup> position is A) |                 |             |                      |



# Relative $\Delta G$ vs. Base Position

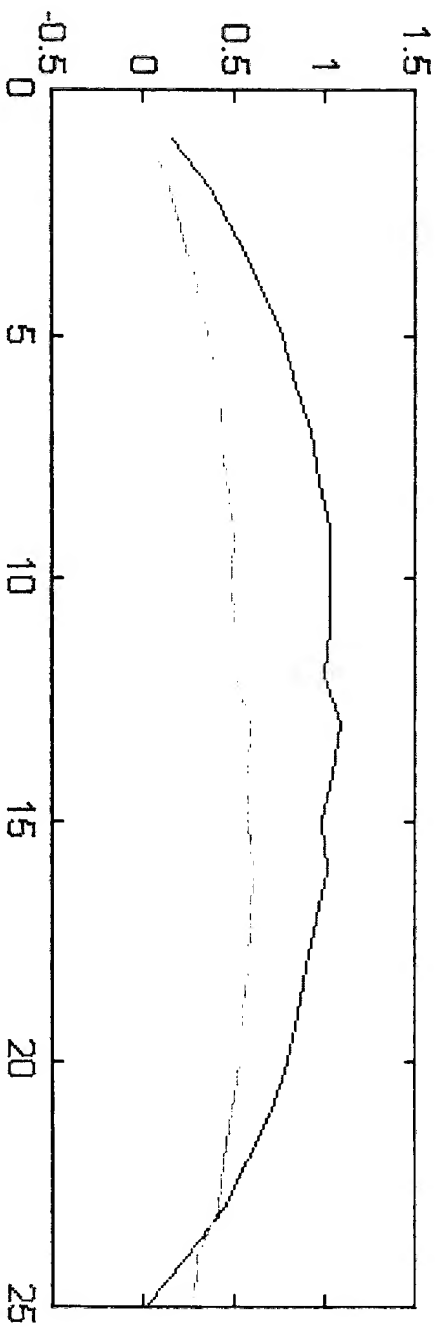


Figure 6A

PM

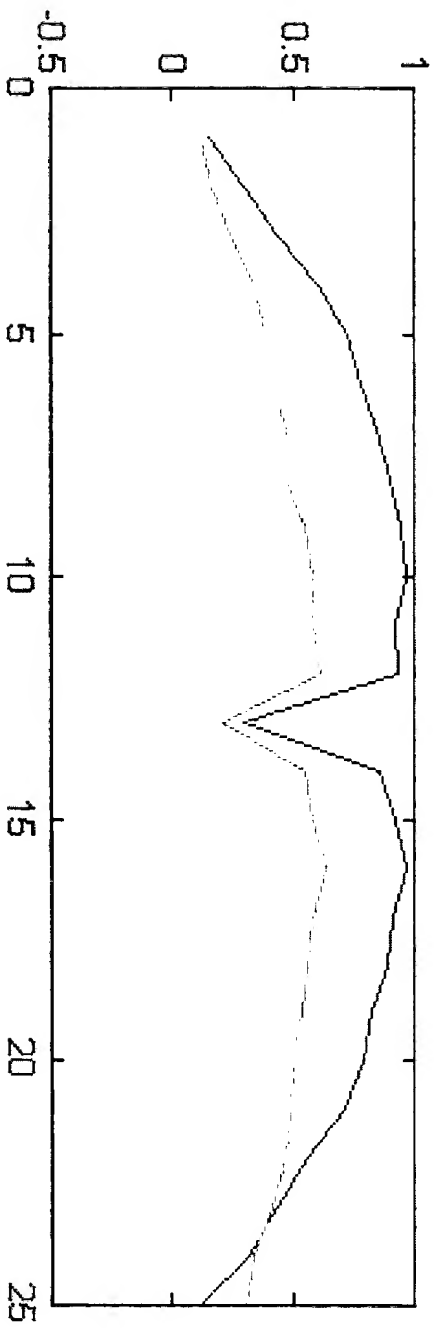


Figure 6B

MM

Base Position in Probe Sequence

U.S. Pat. No. 7,000,000

# Overall Reaction

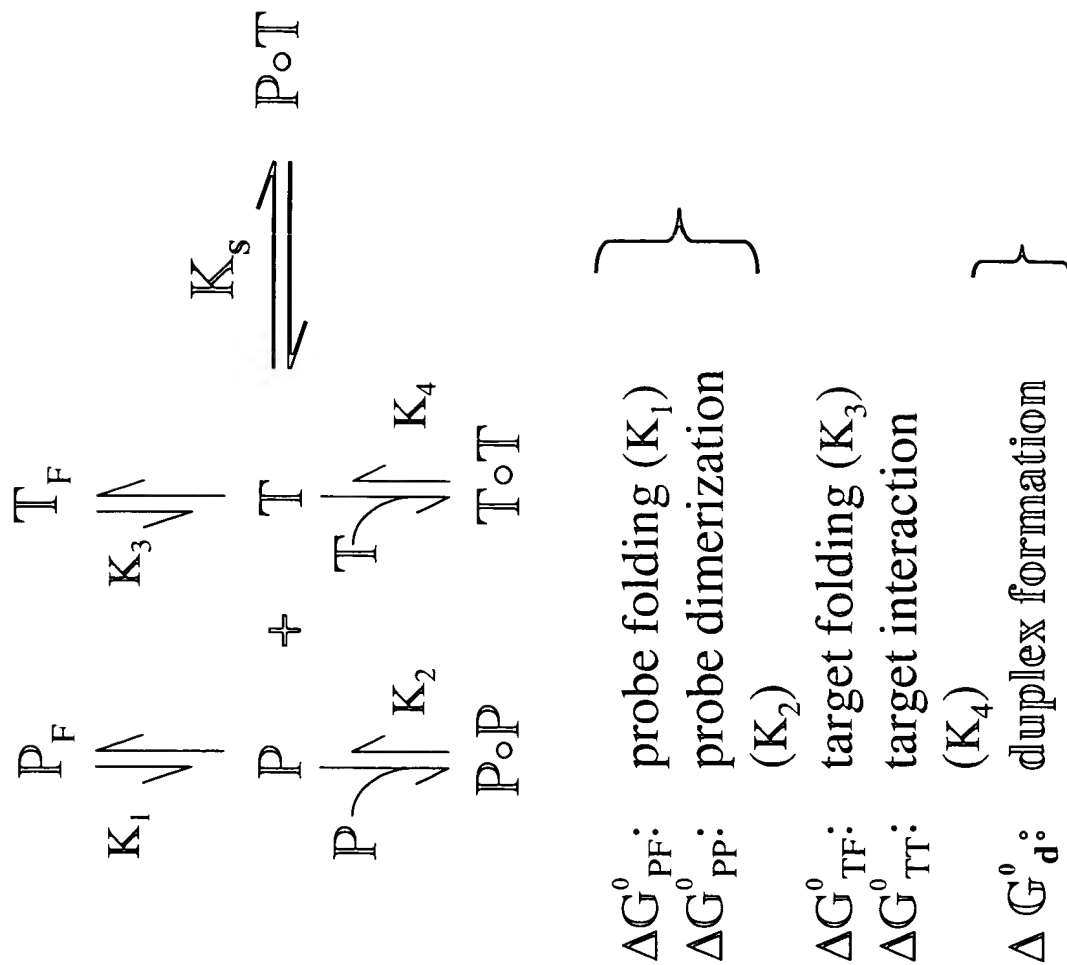


Figure 7



# Concentration Dependence: Slope

Figure 8

$$\ln I = S \cdot \ln C + \ln K_{app}$$

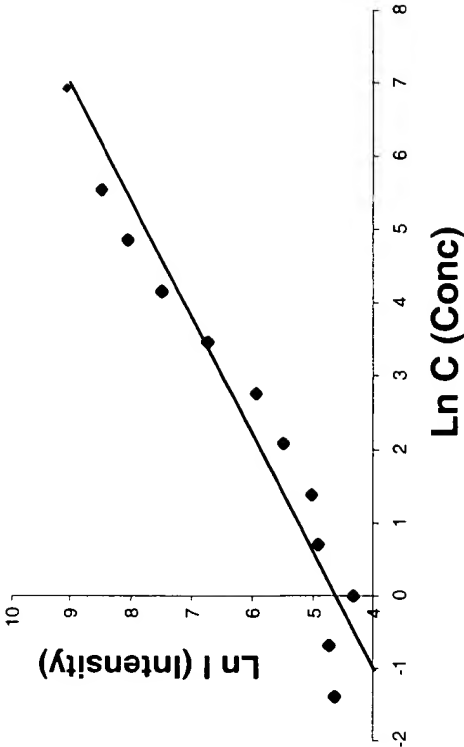
$$I = K_{app} \cdot C^S$$

I: Intensity

K<sub>app</sub>: Apparent Affinity Constant

C: Concentration

S: Empirical Value (0 < S < 1)





# Relationship between Kapp vs. S

- Prediction of Probe Saturation

Figure 9A

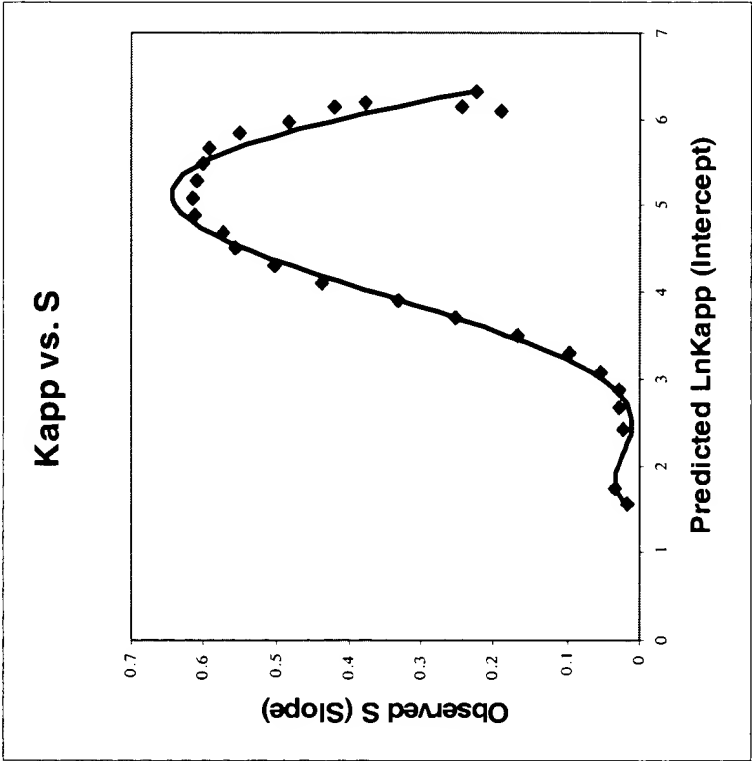
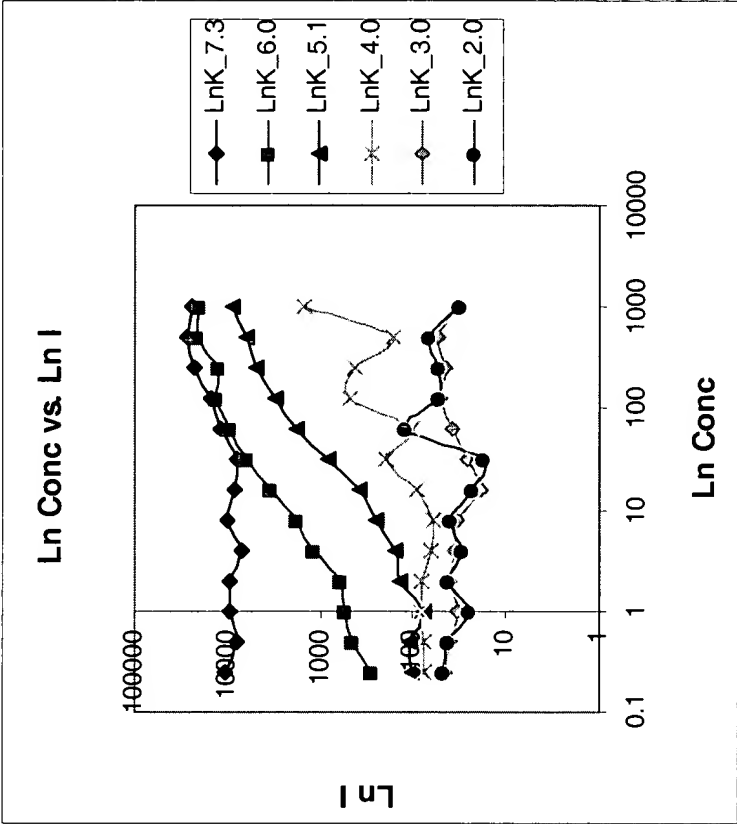


Figure 9B





Candidate Probe  
Sequences



Quality  
Predicator

111

Quality Scores for Each  
Probe

112



Probe  
Selection

113



Selected Probes

Figure 10

09745965-122100  
DOTNET-59654260

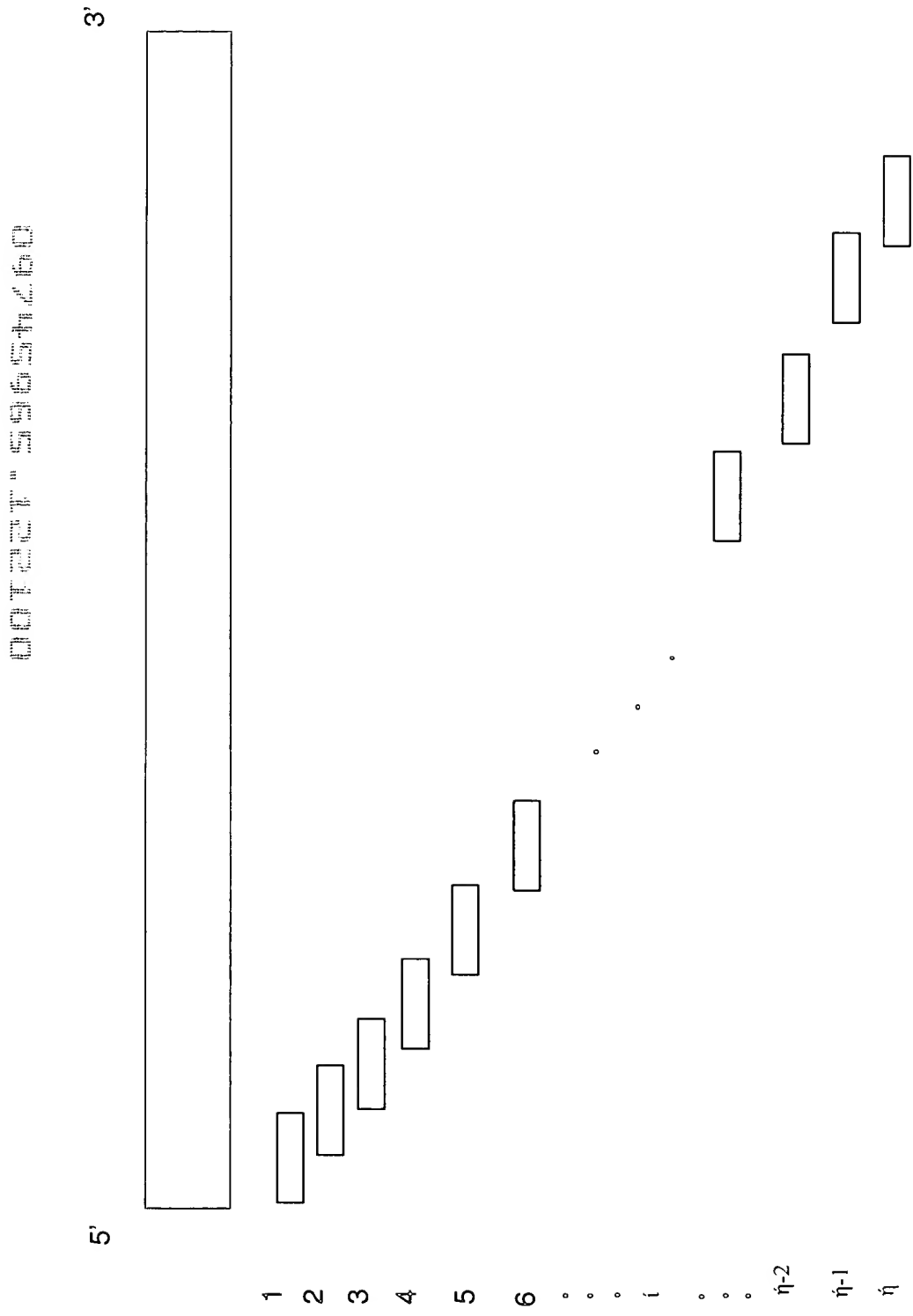


FIGURE 11

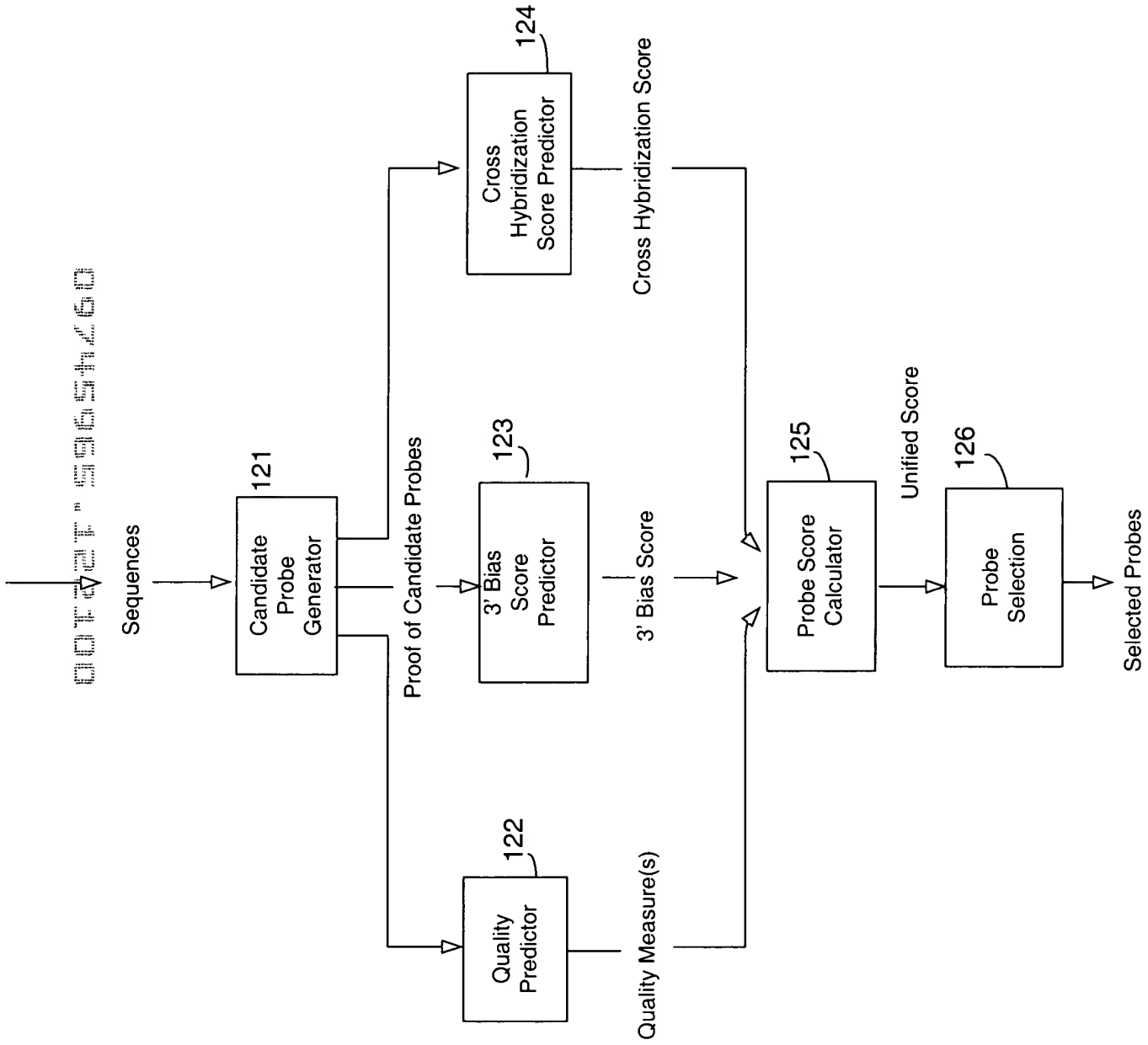


Figure 12

001237" 59654260

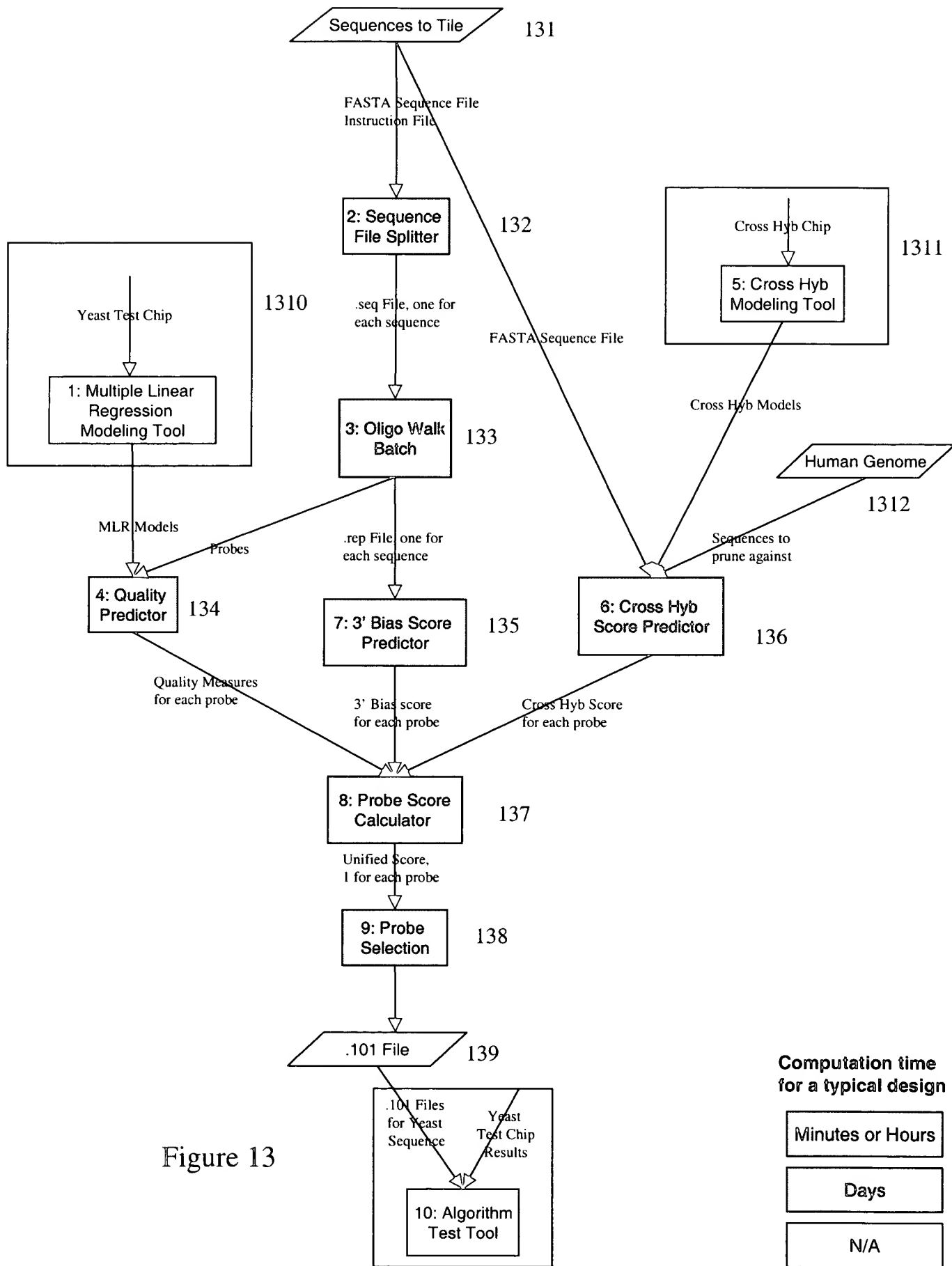


Figure 13

Computation time  
for a typical design

|                  |
|------------------|
| Minutes or Hours |
| Days             |
| N/A              |

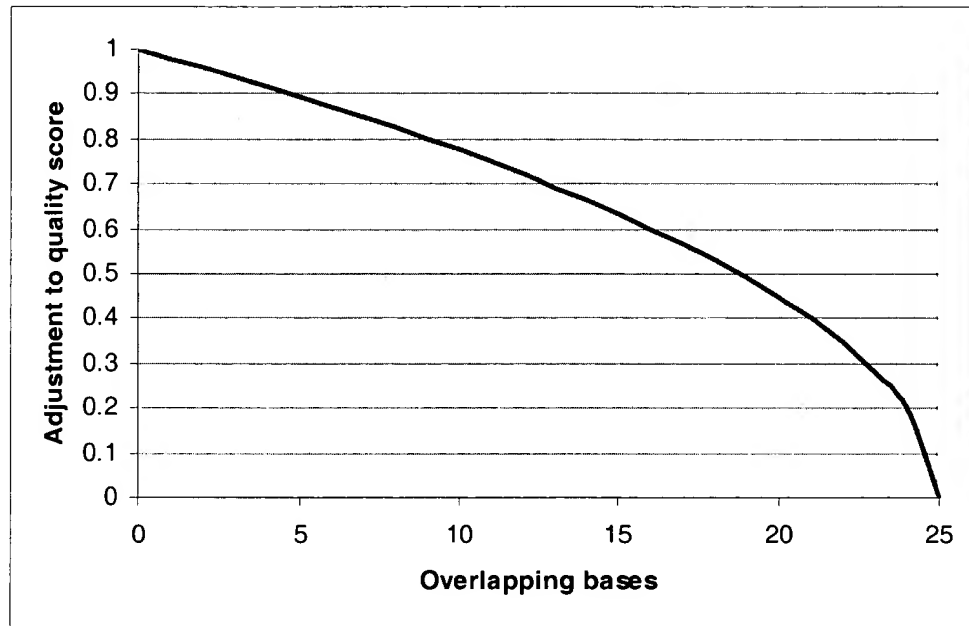
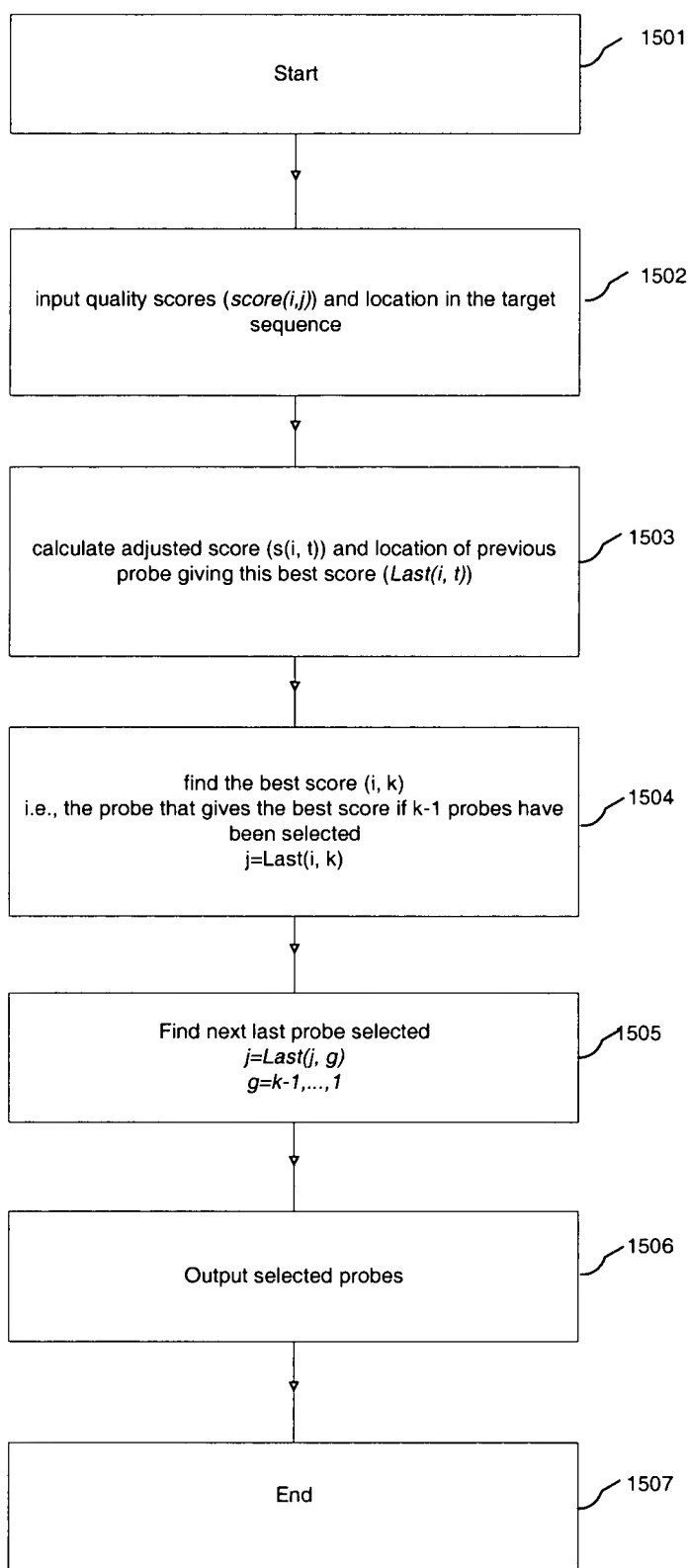


Figure 14



# Latin Square MLR

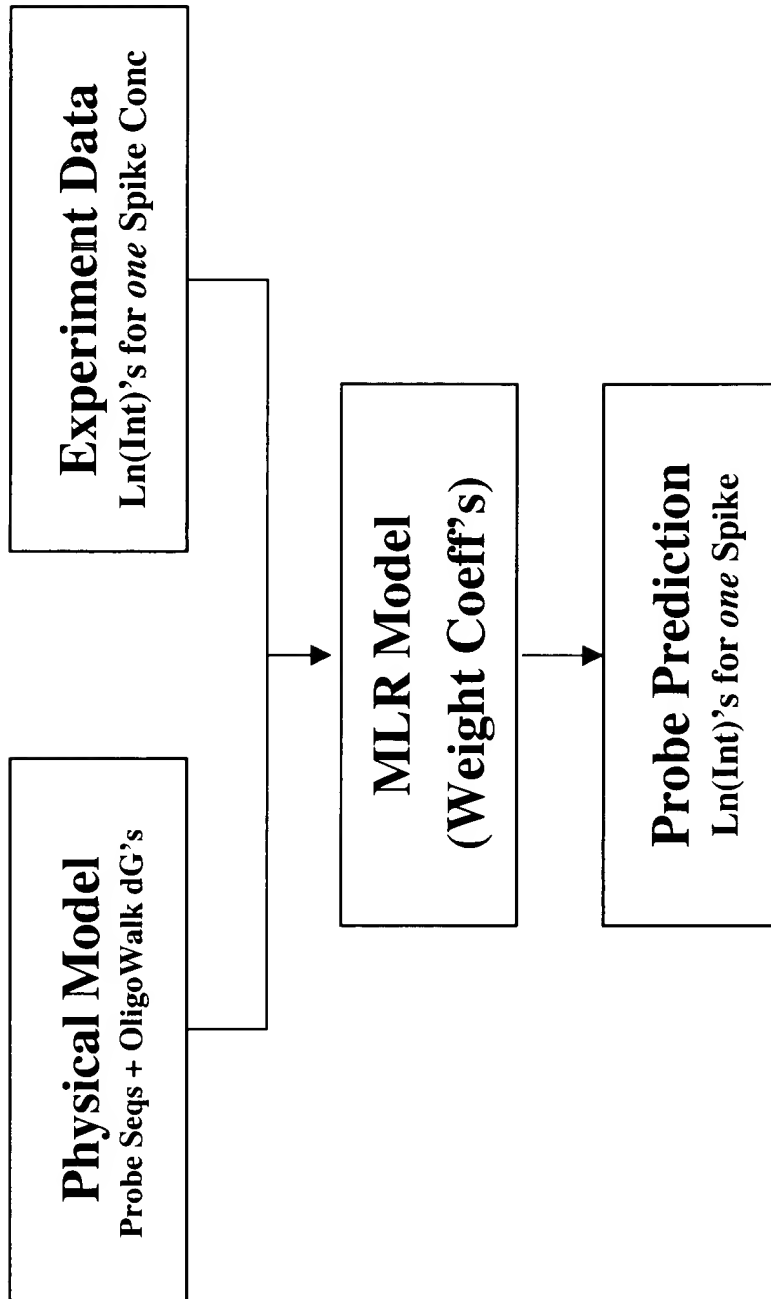


Figure 16



# 112 Yeast Clones Randomly Divided into 14 Groups

## Groups

| 1       | 2       | 3       | 4       | 5       | 6       | 7       | 8       | 9       | 10      | 11      | 12      | 13      | 14      |
|---------|---------|---------|---------|---------|---------|---------|---------|---------|---------|---------|---------|---------|---------|
| YNL259C | YNL037C | YAL038W | YHR044C | YMR127C | YLR377C | YOL064C | YPL209C | YIR034C | YJR148W | YEL046C | YGR185C | YBR166C | YOL165C |
| YEL003W | YDR113C | YLR083C | YJL117W | YNL290W | YOL086C | YJR094C | YFL029C | YMR276W | YML060W | YGR072W | YGL181W | YJL155C | YNL227C |
| YDL235C | YGL105W | YLL043W | YMR116C | YMR228W | YJR019C | YIR026C | YGR040W | YMR294W | YDL188C | YMR203W | YGL213C | YEL036C | YNL228W |
| YEL024W | YDR498C | YBR212W | YPL111W | YPR057W | YOR085W | YLR056W | YPR065W | YPL001W | YGR109C | YGR112W | YOL136C | YJL014W | YMR108W |
| YEL018W | YDL029W | YNL015W | YCL055W | YNR035C | YDL226C | YMR270C | YPR191W | YFL039C | YOL043C | YHR208W | YEL037C | YJL110C | YPL043W |
| YER161C | YKL081W | YDL075W | YFR025C | YCL032W | YBL016W | YBR018C | YMR139W | YNL307C | YLR291C | YIL136W | YHL022C | YFL056C | YLR153C |
| YKL193C | YFR053C | YML098W | YLR354C | YIL154C | YBL068W | YBR057C | YPR035W | YGL148W | YDR088C | YOR099W | YHL014C | YJR155W | YPR074C |
| YPR129W | YFL018C | YOL143C | YPL069C | YBR034C | YHR025W | YER118C | YNL005C | YGL155W | YNR015W | YOR176W | YKR061W | YNL331C | YPL089C |

Figure 17

# Latin Square Experiment

| Exp | Groups |      |      |      |      |      |      |      |      |      |      |      |      |      |
|-----|--------|------|------|------|------|------|------|------|------|------|------|------|------|------|
|     | 1      | 2    | 3    | 4    | 5    | 6    | 7    | 8    | 9    | 10   | 11   | 12   | 13   | 14   |
| 1   | 0      | 0.25 | 0.5  | 1    | 2    | 4    | 8    | 16   | 32   | 64   | 128  | 256  | 512  | 1024 |
| 2   | 0.25   | 0.5  | 1    | 2    | 4    | 8    | 16   | 32   | 64   | 128  | 256  | 512  | 1024 | 0    |
| 3   | 0.5    | 1    | 2    | 4    | 8    | 16   | 32   | 64   | 128  | 256  | 512  | 1024 | 0    | 0.25 |
| 4   | 1      | 2    | 4    | 8    | 16   | 32   | 64   | 128  | 256  | 512  | 1024 | 0    | 0.25 | 0.5  |
| 5   | 2      | 4    | 8    | 16   | 32   | 64   | 128  | 256  | 512  | 1024 | 0    | 0.25 | 0.5  | 1    |
| 6   | 4      | 8    | 16   | 32   | 64   | 128  | 256  | 512  | 1024 | 0    | 0.25 | 0.5  | 1    | 2    |
| 7   | 8      | 16   | 32   | 64   | 128  | 256  | 512  | 1024 | 0    | 0.25 | 0.5  | 1    | 2    | 4    |
| 8   | 16     | 32   | 64   | 128  | 256  | 512  | 1024 | 0    | 0.25 | 0.5  | 1    | 2    | 4    | 8    |
| 9   | 32     | 64   | 128  | 256  | 512  | 1024 | 0    | 0.25 | 0.5  | 1    | 2    | 4    | 8    | 16   |
| 10  | 64     | 128  | 256  | 512  | 1024 | 0    | 0.25 | 0.5  | 1    | 2    | 4    | 8    | 16   | 32   |
| 11  | 128    | 256  | 512  | 1024 | 0    | 0.25 | 0.5  | 1    | 2    | 4    | 8    | 16   | 32   | 64   |
| 12  | 256    | 512  | 1024 | 0    | 0.25 | 0.5  | 1    | 2    | 4    | 8    | 16   | 32   | 64   | 128  |
| 13  | 512    | 1024 | 0    | 0.25 | 0.5  | 1    | 2    | 4    | 8    | 16   | 32   | 64   | 128  | 256  |
| 14  | 1024   | 0    | 0.25 | 0.5  | 1    | 2    | 4    | 8    | 16   | 32   | 64   | 128  | 256  | 512  |

Figure 18

# Latin Square Data Sets from Yeast\_Test\_Hlyb Chips

|                        |         |          |  |
|------------------------|---------|----------|--|
| <i>Lot 1 (9912072)</i> |         |          |  |
| No Background:         | 3 Scans | 14 chips | (530, PMT=701; 570, PMT=701; 570, PMT=600) |
| + Background:          | 3 Scans | 14 chips | (530, PMT=701; 570, PMT=701; 570, PMT=600) |
| <i>Lot 2 (9910426)</i> |         |          |  |
| No Background:         | 1 Scan  | 14 chips | (570, PMT=600)                             |
| No Background:         | 1 Scan  | 14 chips | (570, PMT=600)                             |
| <i>Lot 3 (9910427)</i> |         |          |  |
| + Background:          | 1 Scan  | 14 chips | (570, PMT=526)                             |
| No Background_Rep1:    | 1 Scan  | 14 chips | (570, PMT=526)                             |
| No Background_Rep2:    | 1 Scan  | 14 chips | (570, PMT=526)                             |
| <i>Lot 4 (9913514)</i> |         |          |  |
| No Background:         | 1 Scan  | 14 chips | (570, PMT=526)                             |
| + Background:          | 1 Scan  | 14 chips | (570, PMT=526)                             |
| <i>Lot 5 (9914059)</i> |         |          |  |
| + Background_Rep1:     | 1 Scan  | 14 chips | (570, PMT=526)                             |
| + Background_Rep2:     | 1 Scan  | 14 chips | (570, PMT=526)                             |
| No Background:         | 1 Scan  | 14 chips | (570, PMT=526)                             |

Figure 19

# Bootstrapping

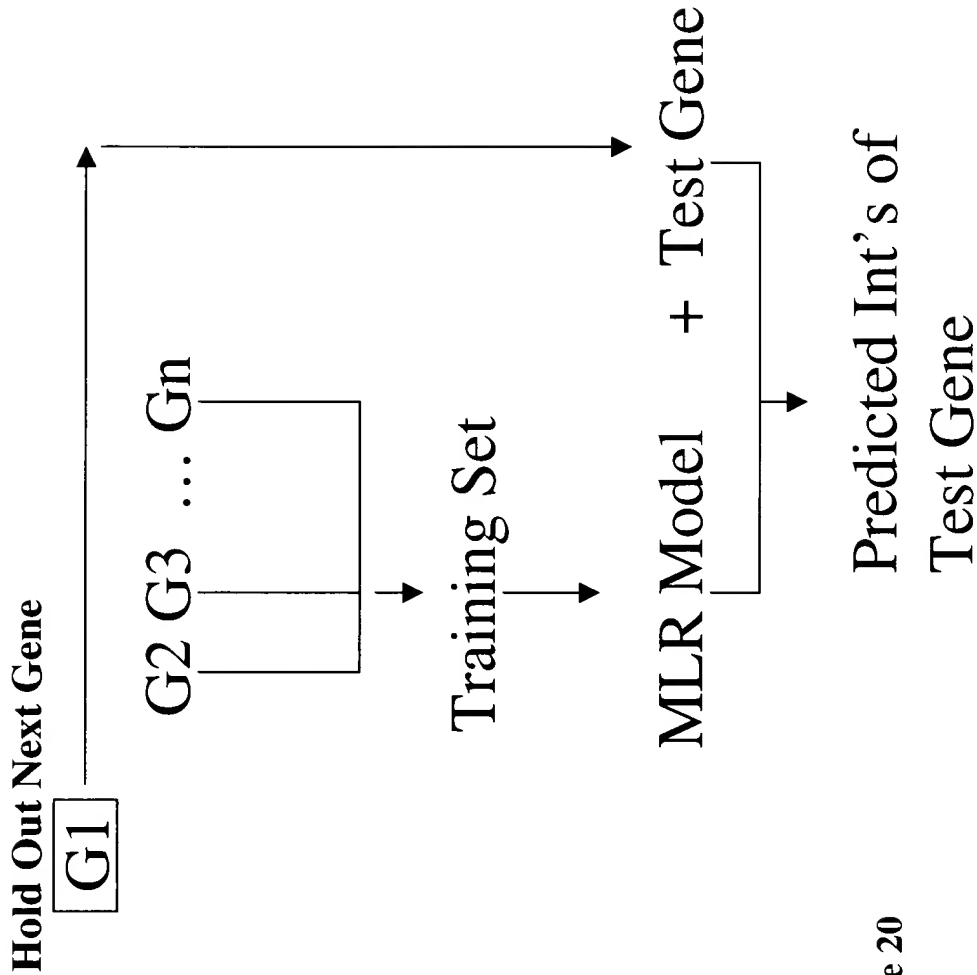
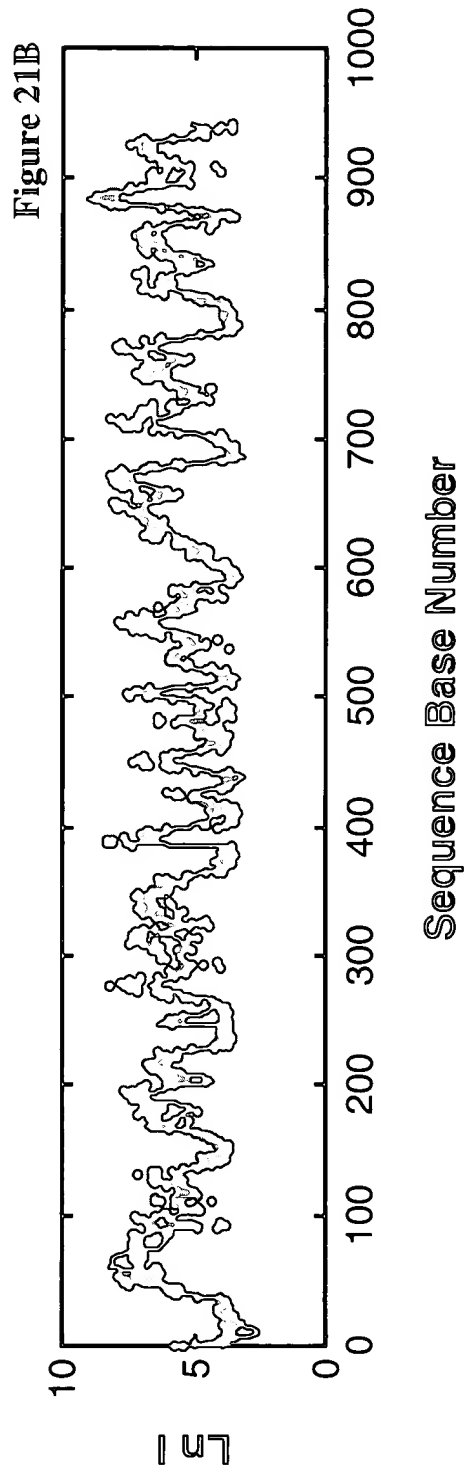
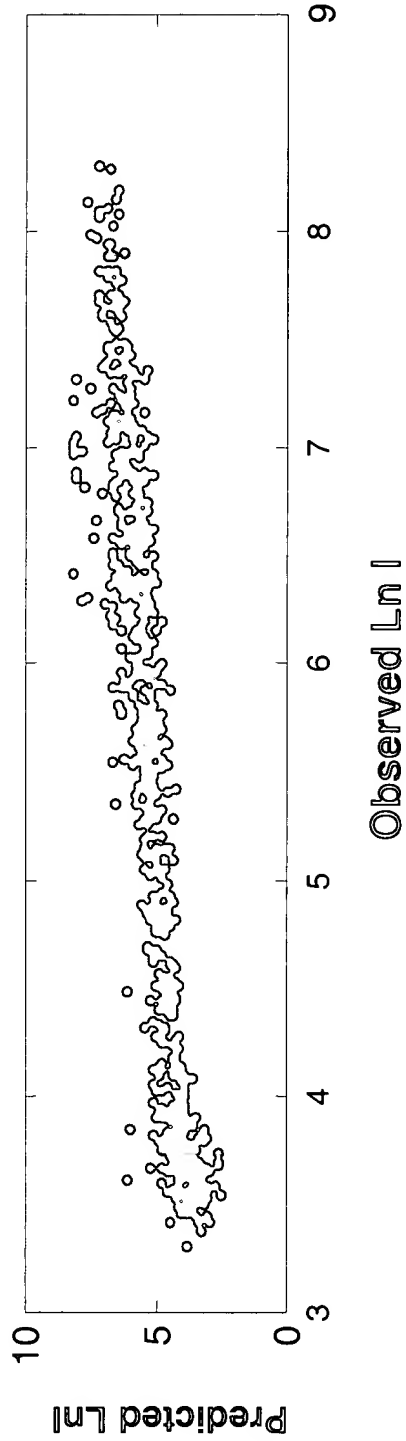


Figure 20



YDR113C  
Figure 21A



007221" 59654/60

YGR109C

Figure 22A

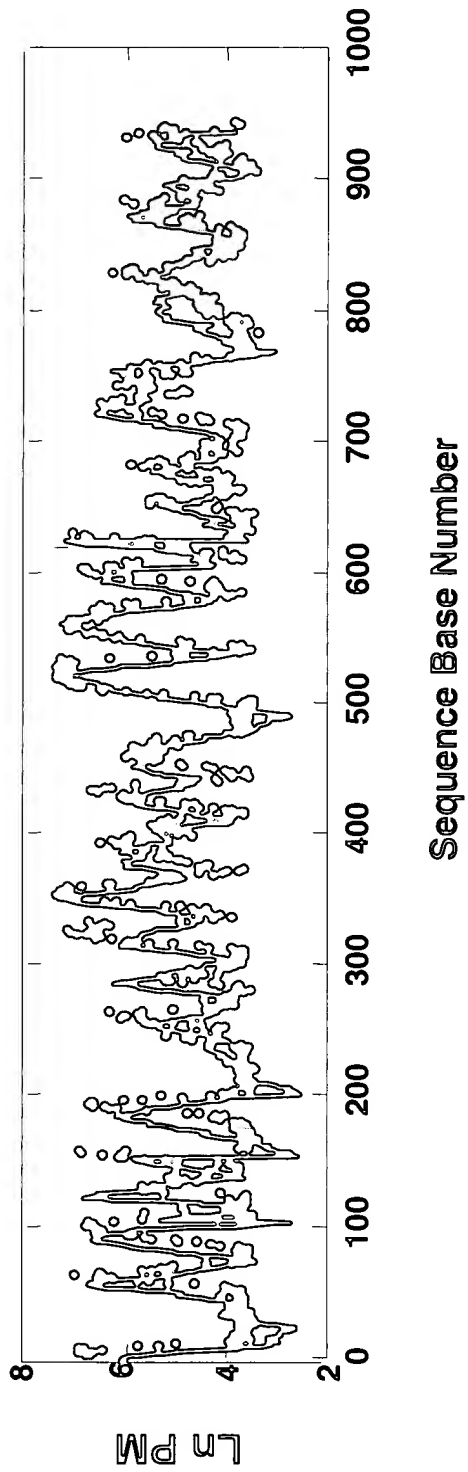
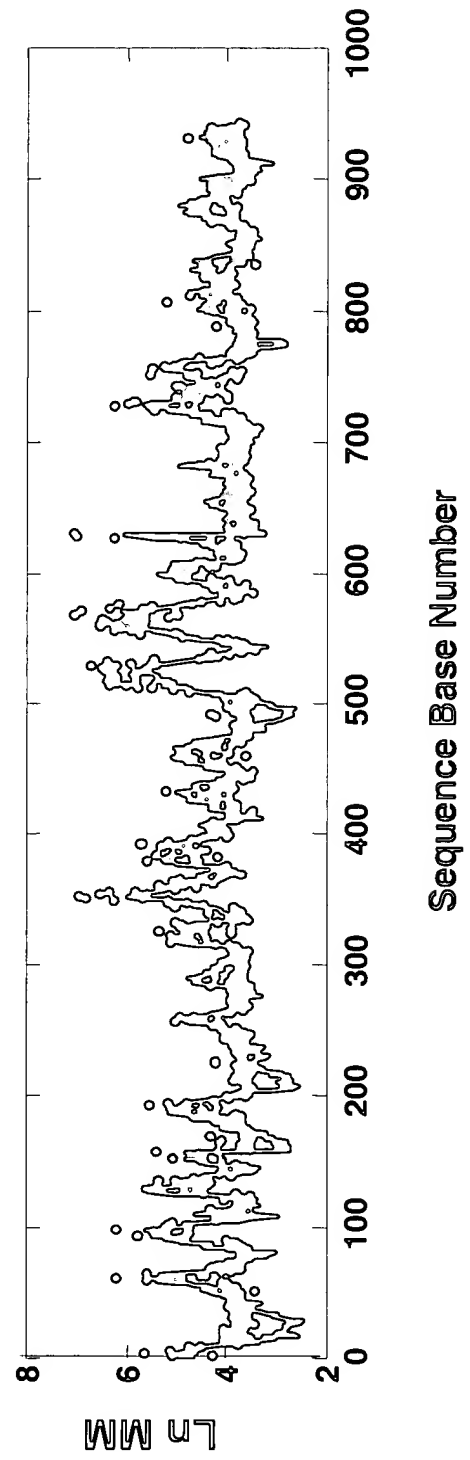


Figure 22B



# Ln(Int) at Different Spike Concentrations

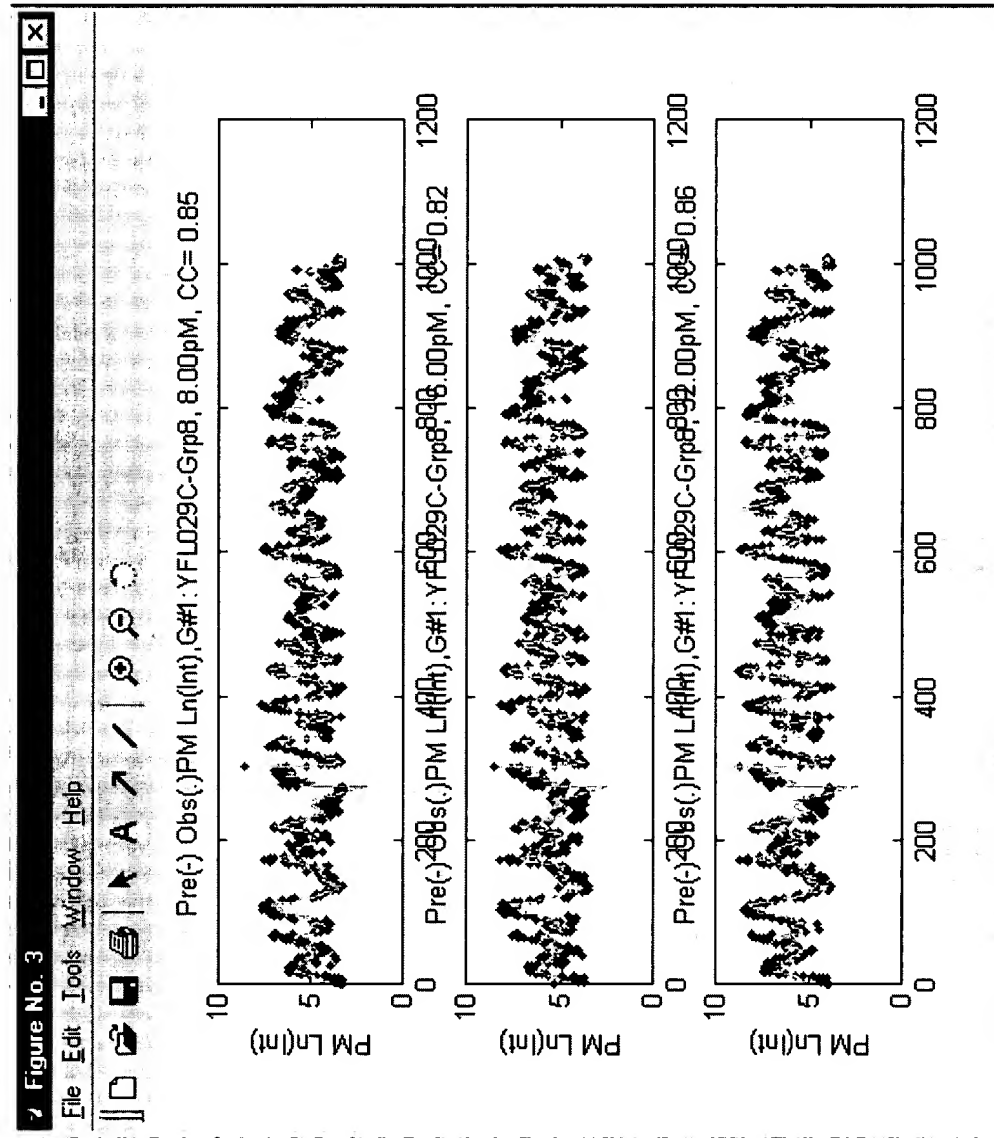


Figure 23

# Correlation between Predicted & Observed $\text{Ln}(\text{Int})$ 's

Title: Methods for Selecting Nucleic Acid Probes  
Inventor: Hubbell  
Attorney Docket No. 3373.1  
Sheet 24 of 30

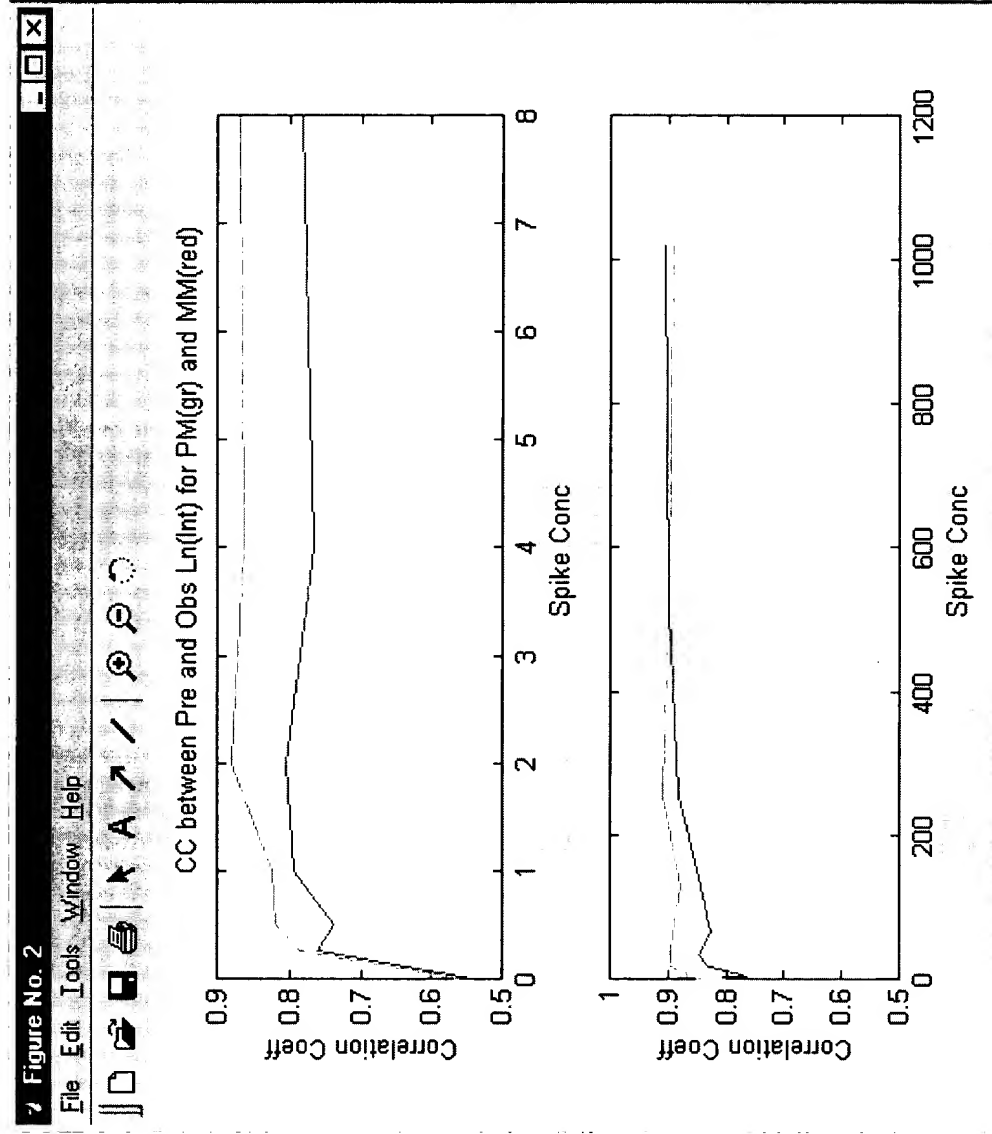


Figure 24



# Negative Control: Gene in Wrong Orientation

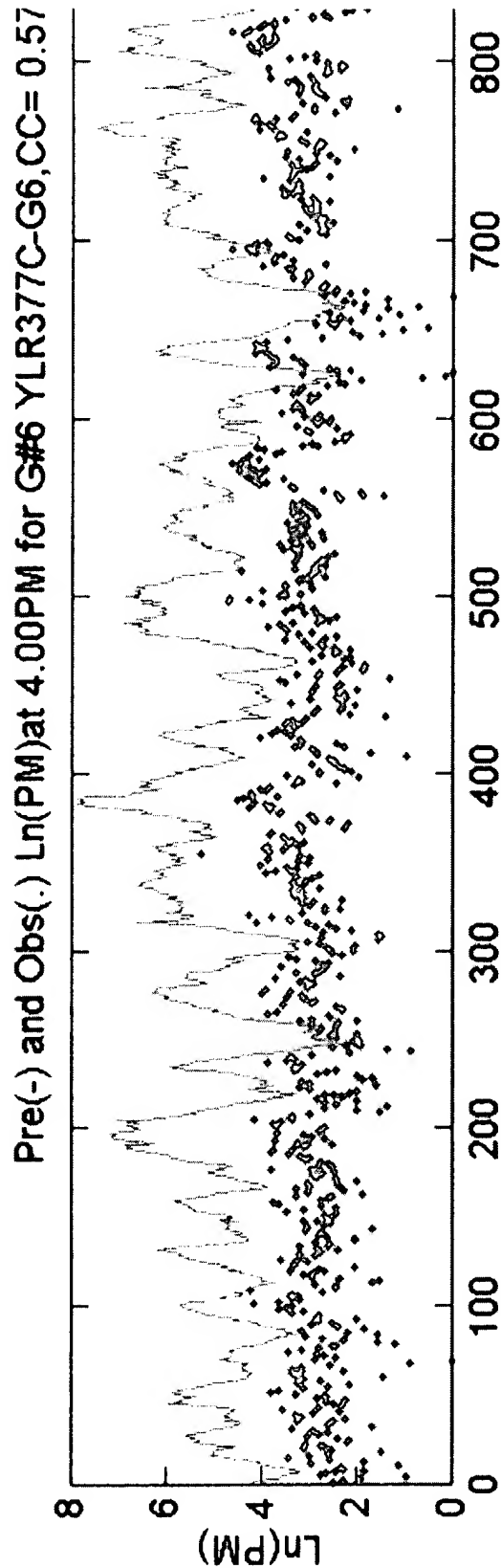
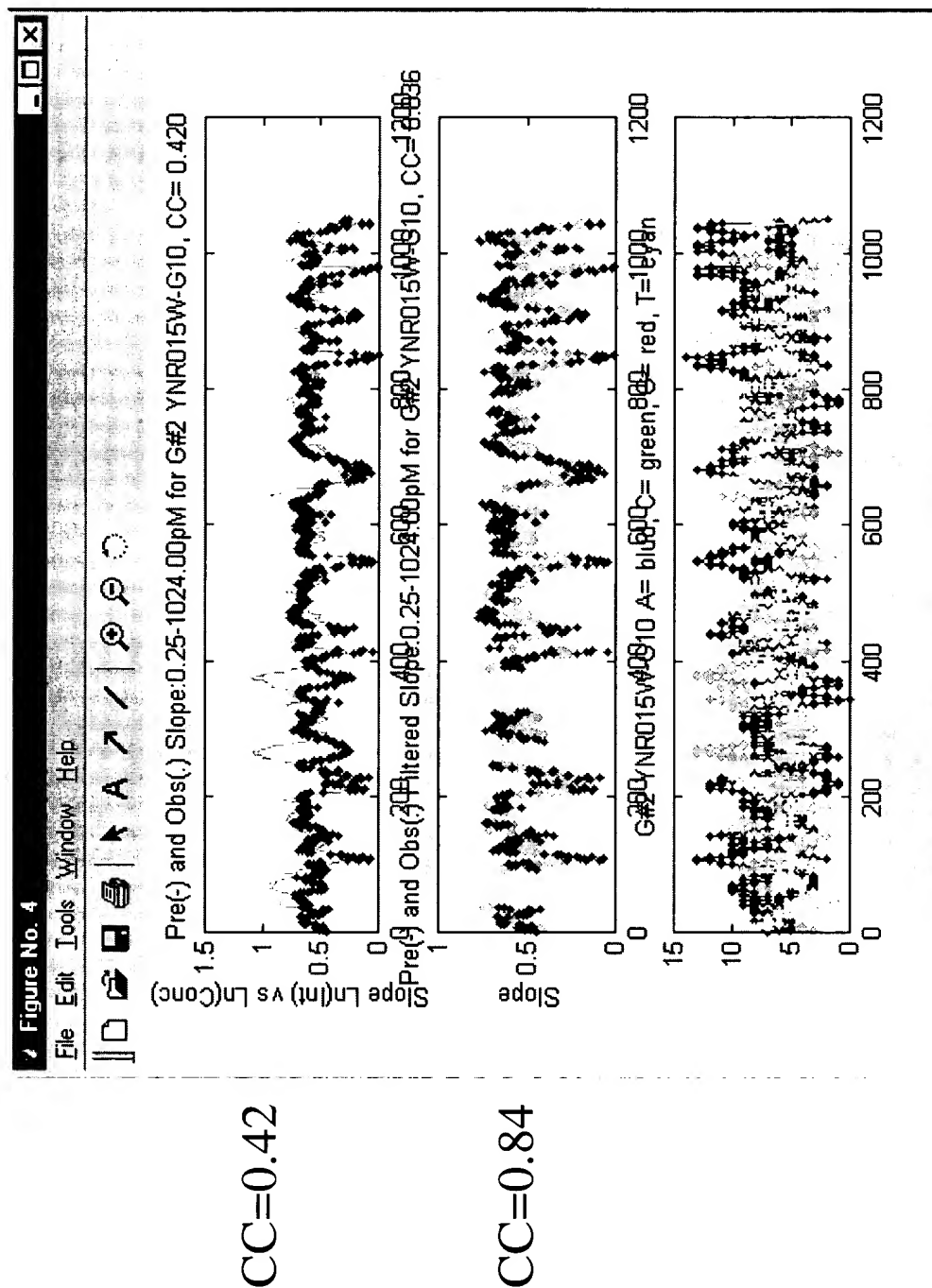


Figure 25

# Predicted Observed Slopes

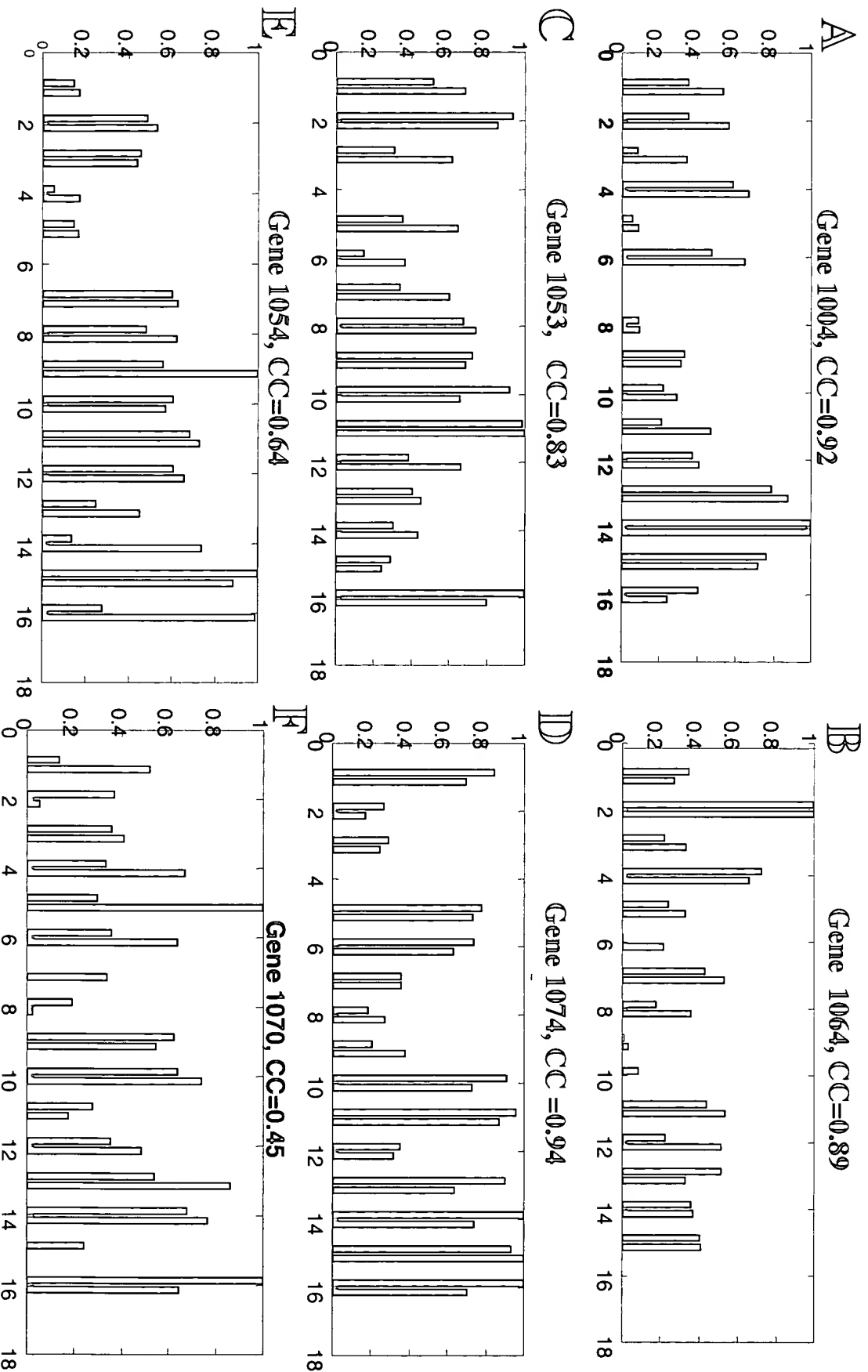


**Figure 26**

# From Yeast to Human

Figure 27

Using Parameters from Yeast Model System to Predict Human U95A



09745965.1.22100

001221" 59554260

# Predictions for Hu\_U95a Probe Sets

Title: Methods for Selecting Nucleic Acid  
Probes  
Inventor: Hubbell  
Attorney Docket No. 3373.1  
Sheet 28 of 30

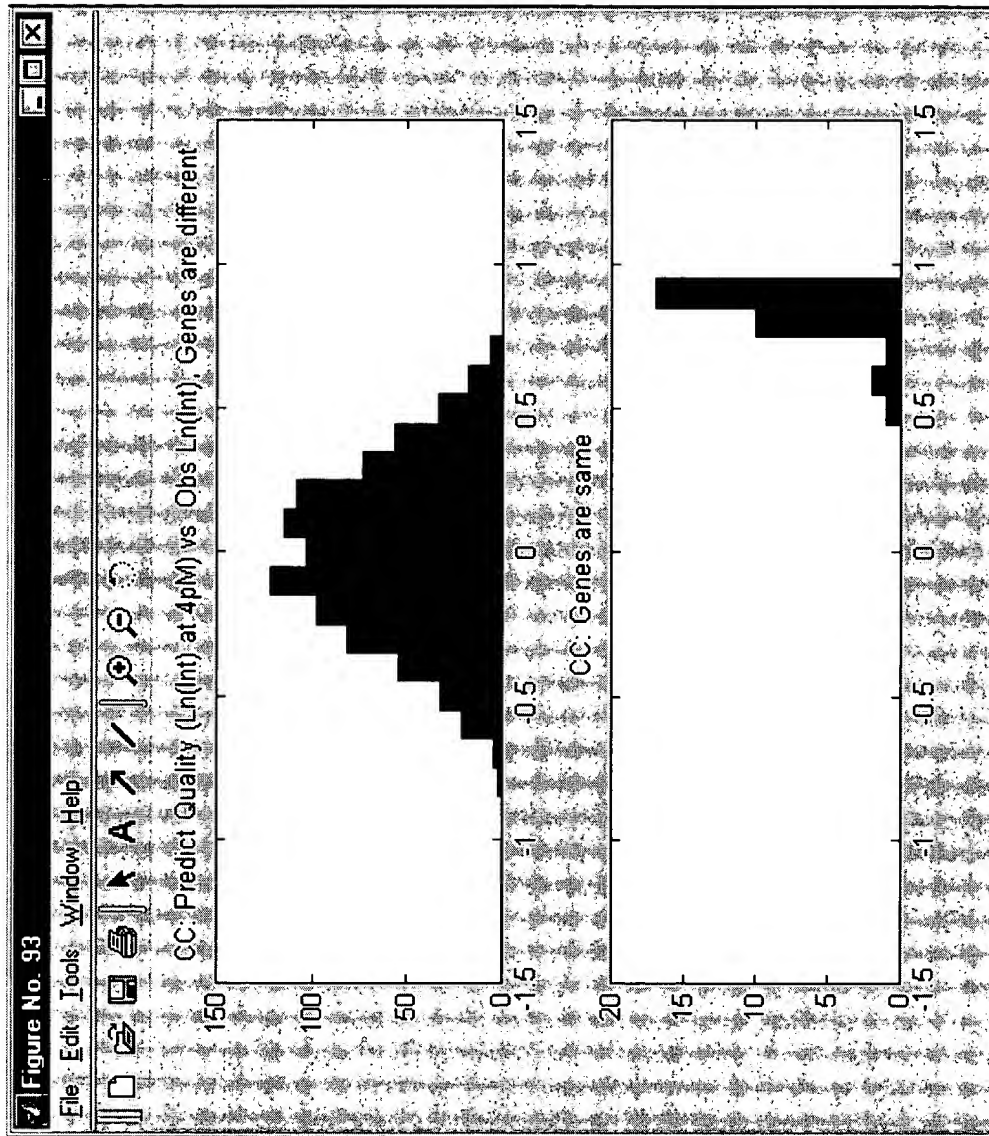


Figure 28

DOT22T" 596544260

# Sixteen Probes Selected By Dynamic Programming Algorithm

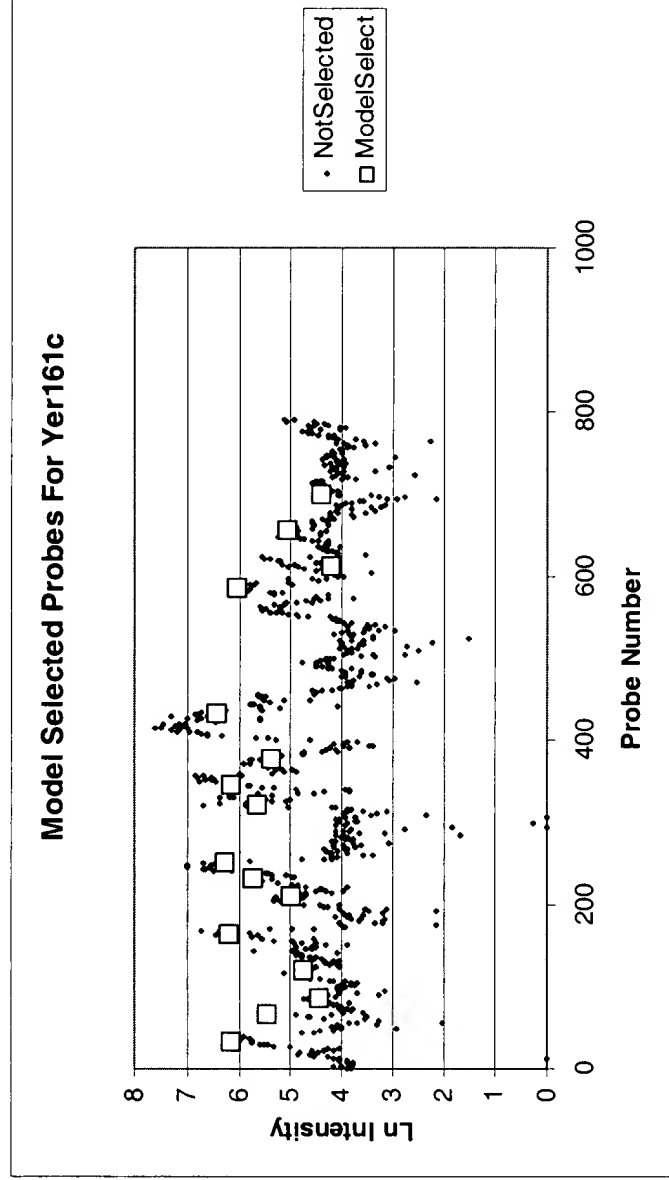


Figure 29

# Comparison of AveDiff Values of all Yeast Test Chip Genes: New vs Random vs Rules Selection

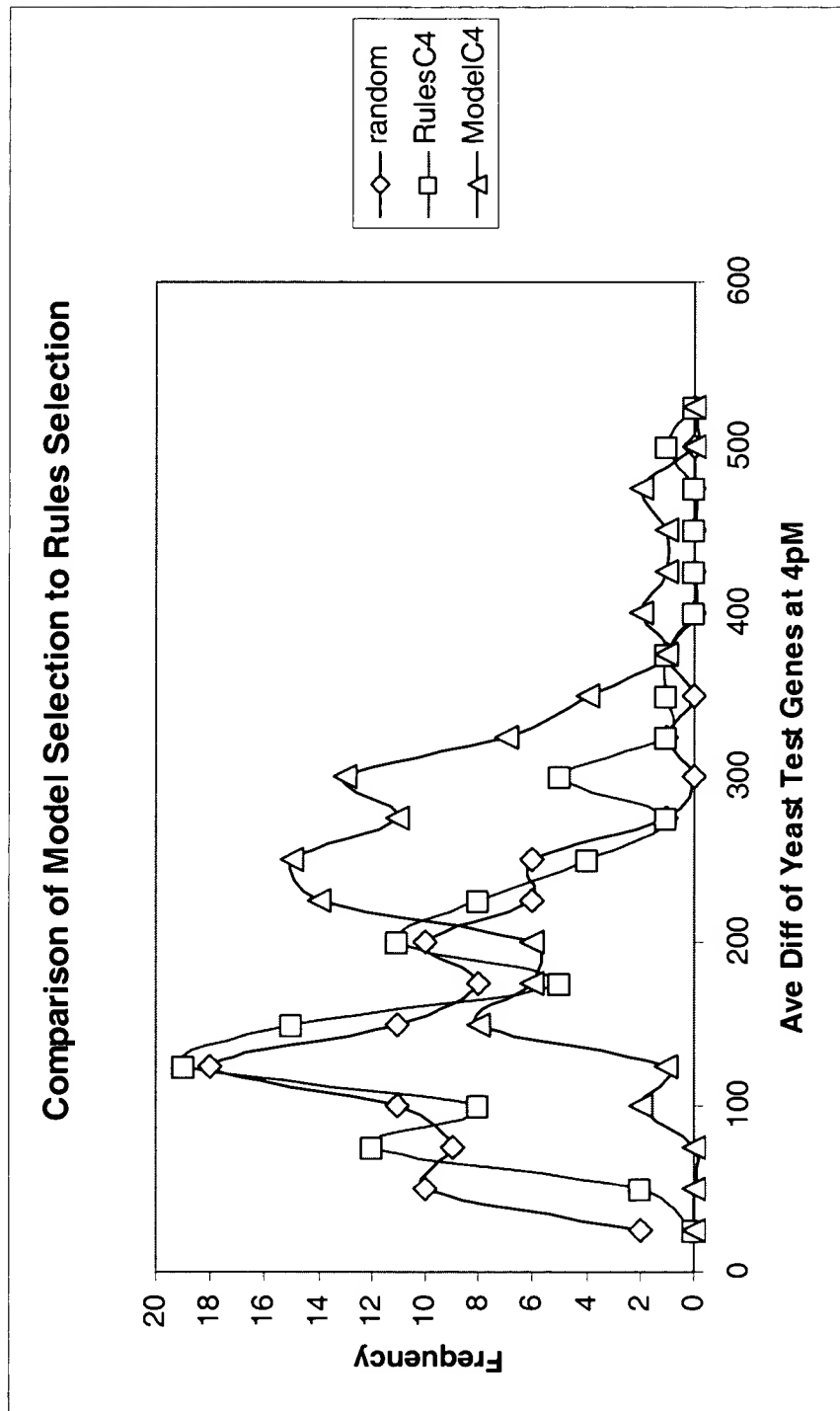


Figure 30